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First detection of a microsporidium in the crucifer pest *Hellula undalis* (Lepidoptera: Pyralidae)—a possible control agent?

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Abstract

For the first time, a microsporidian infection was observed in the pest species *Hellula undalis* (Lepidoptera: Pyralidae) in crucifer fields in Nueva–Ecija, Philippines. Because *H. undalis* is difficult to control by chemical insecticides and microbiological control techniques have not been established, we investigated the biology, the pathogenicity and transmission of this pathogen found in *H. undalis*. An average of 16% of larvae obtained from crucifer fields in Nueva–Ecija displayed microsporidian infections and 75% of infected *H. undalis* died during larval development. Histopathological studies revealed that the infection is initiated in the midgut and then spreads to all organs. The microsporidium has two different sporulation sequences: a disporoblastic development producing binucleate free mature spores and an octosporoblastic development forming eight uninucleate spores. These two discrete life cycles imply its taxonomic assignment to the genus *Vairimorpha*. The size of the binucleate, diplokaryotic spores as measured on Giemsa-stained smears was $3.56 \pm 0.29\,\mu m$ in length and $2.18 \pm 0.21\,\mu m$ in width, whereas the uninucleate octospores measured $2.44 \pm 0.20 \times 1.64 \pm 0.14\,\mu m$. The microsporidium was orally and probably also transovarially transmitted. Laboratory infection tests by feeding spores on artificial diet to 3rd instars resulted in 100% infection with a final mortality of 80%. Most larvae died during larval development and 40% of them died 3 days after spore ingestion. This microsporidium shows potential for microbiological pest management.

Keywords: *Hellula undalis*; Cabbage webworm; Microsporidia; *Vairimorpha* sp.; Biological control; Pyralidae; Crucifer pests

1. Introduction

Hellula undalis (F.) (Lepidoptera: Pyralidae), the cabbage webworm, is a serious pest on crucifer crops in the tropics and subtropics. It is also found in temperate regions, but infestations are transitory because the species does not develop at temperatures below 20 °C (Sivapragasam et al., 1994). While enclosed in a loose web, larvae feed on young leaves and bore inside the growing tip. Here they cause major damage especially to seedlings (Sivapragasam and Chua, 1997a; Mewis et al., 2001a). Feeding damage by a single larva may result in termination of plant development and death or the formation of multiple shoots. In India yield losses of 30% due to *H. undalis* are reported when no chemical insecticides or other control measures are applied (Srihari and Satyanarayana, 1992). Yield losses up to 100% within 3 weeks have been documented in untreated crucifer fields in the Philippines during the dry season (Mewis et al., 2001b). *H. undalis* was first observed in crucifer fields in the Philippines near Los Banos in 1990 (Rejesus and Javier, 1997). Since its discovery, it has increased in numbers and is now considered as one of the most serious pests of crucifers in the Philippines, along with *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and *Spodoptera litura* (F.) (Lepidoptera: Noctuidae).

Chemical control of *H. undalis* is not very effective because the larvae are shielded in a loose web in the

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growing point (shoot) and have probably acquired resistance to insecticides (Sivapragasam and Aziz, 1990). Because alternative biological or integrated control methods are urgently needed, we searched for natural antagonists of *H. undalis* in the Philippines. Although several larval and egg parasitoids (e.g., *Apanteles* sp., *Bracon gelechiae* Ashm., *Microchelonus blackburni* Cam., and *Trichogramma minutum* Riley), and pathogens (*Bacillus thuringiensis* Berliner, granulovirus, and nucleopolyhedrovirus) are described from *H. undalis* in Africa and other regions of Asia (AVRDC, 1987; Waterhouse and Norris, 1989; Sivapragasam and Aziz, 1990; Battu, 1991; Sivapragasam and Chua, 1997b), none of them was found in the Philippines in this species during our observation from 1998–2001. However, we observed the first microsporidian infection of *H. undalis* in a population infesting cruciferous crops in the Central Luzon region.

As a phylum, Microsporidia with more than 1000 species in 144 genera has a broad range of host organisms (Moser et al., 2000), and most microsporidian species are known from insects. Thus, they are the most important "protozoan-like" pathogens of insects. Microsporidia usually infect their hosts after ingestion via polar filament extrusion into epithelial cells of the midgut (Kurtz et al., 2000). Frequently, the infection spreads from the midgut to susceptible "target" tissues depending on microsporidian species and host (Hoch et al., 2000; Kurtz et al., 2000). There is some evidence that hemocytes carry spores and mediate the spread of infection in the insect's body (David and Weiser, 1994; Kurtz et al., 2000). Identification of microsporidian species is commonly based on the morphology, biochemistry, and germination of spores, the life cycle, the host or host range. More recently, sequencing of rDNA is being used.

Microsporidia are natural regulating factors of some economically important insect pest populations (Onstad and Carruthers, 1990). Tanada and Kaya (1993) stated that they are the most promising protozoa for microbiological control. At least one microsporidium, *Nosema locustae* Canning, has reached the commercial stage in development and has been used in biological control of grasshopper (Henry and Oma, 1981). However, there are still controversial opinions on the possible use of microsporidia in microbiological control of different pest insects. Because of the growing interest in research to use microsporidia in pest control and due to problems in chemical control of the cruciferous pest *H. undalis*, the detection of a microsporidiosis in this species is of special interest. Therefore, we investigated the prevalence of microsporidian infections in natural populations of *H. undalis*. Furthermore, we studied the transmission and the pathogenicity of the microsporidium found in *H. undalis* to estimate its potential use as a microbiological control agent.

2. Materials and methods

2.1. Insect rearing

Hellula undalis larvae were obtained from crucifer fields in the province Nueva–Ecija (Central Luzon) in the Philippines. A microsporidian free-stock culture was established at the Laboratory of Central Luzon State University (CLSU) by using eggs from uninfected adults. Eggs were obtained from *B. campestris* L. subsp. *chinensis* var. Black Behi (pak choi). Abdomens of adults were checked for microsporidian infection by dissecting tissues, such as fat body and gut, which were examined for the presence of spores and vegetative stages by using phase contrast microscopy at 400 and 1000×. The examination for microsporidian infection in adults was absolutely necessary because the offspring from unchecked adults often died due to microsporidian infection. Larvae of *H. undalis* were reared on *Brassica chinensis* plants in a cage constructed from mesh screen (0.5 mm mesh wide) at 30 ± 2 °C and $85 \pm 10\%$ relative humidity (RH). Additionally, beginning with the 2nd instar, larvae were separately reared on an artificial diet in closed plastic containers (100 ml volume). The artificial diet, a commercial formula developed for *Spodoptera* spp., was obtained from Bioserv (Frenchtown, NJ, USA) and enriched with autoclaved *B. chinensis* aqueous extract at a 1:1 ratio. Cellulose tissue was added into rearing cages to facilitate pupation. Pupae were collected and kept separately from larvae. Emerged adults were fed a 10% (v/v) honey solution.

2.2. Microsporidian prevalence in the field

Studies on the prevalence of microsporidian infection were carried out in Nueva–Ecija. Infection rates for *H. undalis* populations in *B. chinensis* fields (pak choi) were determined from four samples of 100, 3rd–5th instar larvae collected at the following locations and dates: San Jose, 10 November 2000 and 20 December 2000; Matingkis, 11 December 2000, and CLSU-Campus, 5 January 2001. Larvae were kept separately in plastic containers on artificial diet (1 cm cube) at the laboratory as previously described. Every 2 days, the developmental stage and mortality of each *H. undalis* were recorded until adult emergence. During larval development, the artificial diet was replaced every second day. All used facilities and materials were sterilized with 70% ethanol and following treatment by bleach (5.25% sodium hypochlorite) and water. Tissues from dead larvae and abdominal tissues from emerged adults were examined for the presence of microsporidian spores and vegetative stages in wet mounts by using phase contrast microscopy at 400 and 1000×.

2.3. Production of microsporidia

Samples of microsporidian spores used for infection of host larvae were originally obtained from heavily infected *H. undalis* larvae collected in fields in Nueva–Ecija. They were stored in a water suspension at 4 °C for a maximum of 1 day. *H. undalis* larvae, 3rd instar, were fed spores according to the method of Solter et al. (1997), in which spores are spread on the surface of artificial diet. The larvae were maintained on the same artificial diet as previously described at 30 ± 2 °C and $85 \pm 10\%$ RH. Heavily infected live and dead larvae containing environmental spores were collected and frozen at −4 °C (up to 1 month) for production of standard spore suspensions. Defrosted larvae were macerated and grounded by using a mortar and pestle by adding distilled water. The resulting crude suspension of microsporidian spores was filtered (0.1-mm mesh size) to remove larval tissues. The spore suspension was diluted with deionized water and the concentration was determined with a hemocytometer. A standard suspension of 20×10^3 spores/ml was kept at 4 °C until used the next day.

2.4. Histological examination

Studies on the histopathology and life cycle of the microsporidium of *H. undalis* were conducted at the Federal Biological Research Centre for Agriculture and Forestry (BBA), Institute for Biological Control in Darmstadt, Germany. About 60 larvae, 1st–3rd instar, which have been perorally infected with 50 μl (1000 spores) of the standard spore suspension on artificial diet, were used for histological examination. Larvae at different stages of infection were dissected for light microscopy. Different tissues were examined for microsporidian stages in wet mount preparations by phase contrast and in Giemsa-stained smears. Others were fixed with Dubosq–Brazil alcoholic Bouin's and embedded in histosec (Merck, Darmstadt Germany). Sections cut at 4–6 μm were stained with Heidenhain's iron hematoxylin (Merck, Darmstadt Germany) and counterstained with erythrosin. Measurements of the spores were conducted on digital micrographs with Soft Imaging System, analySIS 3.0.

2.5. Host infection experiment

The infectivity and pathogenicity of the microsporidium were tested in an infection experiment with 3rd instar larvae of *H. undalis*. Single larvae were kept in plastic containers on artificial diet (1-cm cube) as previously described at 30 ± 2 °C and $85 \pm 10\%$ RH. The artificial diet cubes offered for larval infection had been previously treated with the standard spore suspension, 50 μl each (ca. 1000 spores). Water was applied on artificial diet in the controls. The test was repeated four times, 40 larvae in each repetition (20 treated and 20 untreated). The mortality and development stage of *H. undalis* were recorded every 2 days until adult emergence. Also every 2 days the artificial diet was replaced during larval development. Used facilities and materials were sterilized with 70% ethanol followed by bleach. Dead larvae and abdomens of adults were examined for microsporidian stages by using phase contrast microscopy. Additionally, ovaries of females were examined for eggs. Data were analyzed for statistical differences among mortality of treatments by using repeated measures analyses of variance (RMANOVA). Statistical tests were performed with the software package SYSTAT 9.0 (SYSTAT 9.0 Handbook, 1999).

3. Results

3.1. Microsporidian prevalence in the field

Microsporidian infections of *H. undalis* were found in all field sites (Table 1). An average of 16% of larvae (3rd–5th instar) obtained from the field died in the laboratory during development due to microsporidiosis. Approximately 3% of larvae died during development due to unknown factors. Of all infected larvae, 75% died before pupation, whereby highest mortality was observed in the 5th instar including prepupae. The percentage mortality of *H. undalis* stages was: 13% in 3rd, 26% in 4th, 36% in 5th instar (13% prepupae), and 25% in pupae. Uninfected larvae completed metamorphosis and adults emerged within 7–17 days. The sex ratio, females:males, was $1{:}0.88 \pm 0.004$. No microsporidia were found in emerged adults.

3.2. Histological examination

Heavy microsporidian infections were detected in different larval tissues using light microscopy. The midgut cells were heavily infected, but the infection also spread to other tissues, such as fat body, Malpighian tubules, hypodermis, muscle cells, and gonads. The presence of the microsporidium in the gonads suggested that the pathogen is probably transmitted via the transovarial (vertically) route, not only peroral (horizontally).

Table 1
Collection sites of *H. undalis* and mortality by microsporidian infection

Collection site	Percentage mortality of *H. undalis* by microsporidian infection ($n = 100$, each)		
	Total	Larvae	Pupae
San Jose	9	3	6
San Jose	27	22	5
Matingkis	17	14	3
CLSU-Campus	11	9	2
Average	16.0 ± 7.0	12.0 ± 7.0	4.0 ± 1.6

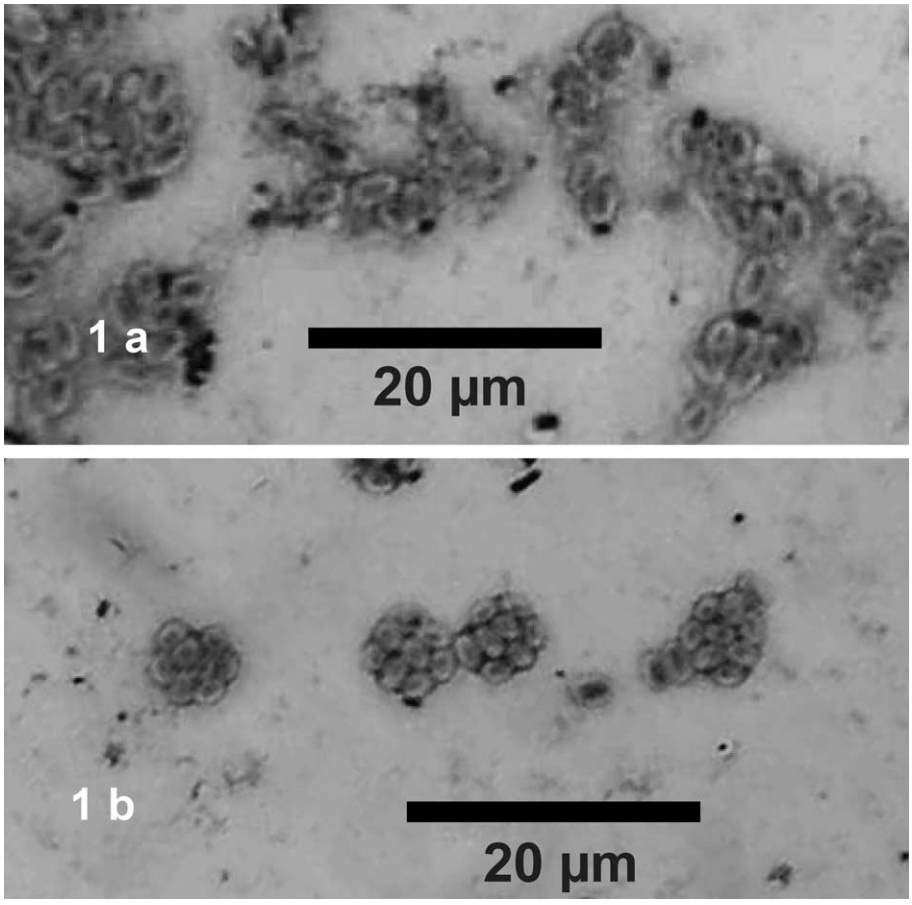

Fig. 1. Giemsa-stained smears of (a) free ovoid binucleate microsporidian spores (diplokaryotic spores), (b) four eight-spore groups of uninucleate microsporidian spores (octospores).

Studies on the life cycle showed that there is a disporoblastic and an octosporoblastic development of the microsporidium. Mature free spores formed via the disporoblastic development are binucleate (diplokaryotic spores; Fig. 1a). The octosporoblastic development results in the production of eight uninucleate spores in a sporophorous vesicle (octospores; Fig. 1b). The size of the ovoid diplokaryotic spores as measured on Giemsa-stained smears was $3.56 \pm 0.29\,\mu m$ in length and $2.18 \pm 0.21\,\mu m$ in width ($n = 50$). Octospores in the vesicle were also ovoid but smaller with $2.44 \pm 0.20 \times 1.64 \pm 0.14\,\mu m$ ($n = 50$) (Table 2).

3.3. Host infection experiment

A significantly higher mortality of *H. undalis* was observed after peroral infection with microsporidian spores as compared to the control (RMANOVA: $F = 1.95$, $df = 6$, and $p < 0.0001$), whereby in treated larvae the mortality increased significantly with time. Already on the third day, the mortality of treated 3rd instar larvae was about $35 \pm 9\%$ (Fig. 2) and only an average of $20 \pm 3\%$ completed the development to adults. Most larvae died during larval development (83%) and only 17% died during the pupal stage. Mean mortality of larval stages was: 56% 3rd, 11% 4th, and 16% 5th instar (prepupae: 10%). In this experiment most larvae died at the 3rd instar. The examination of emerged adults and dead larvae from treated 3rd instar by light microscope showed that vegetative microsporidian stages as well as mature spores were always present in different tissues such as fat body, midgut, and muscle cells. Therefore, 100% of 3rd instar larvae became infected after feeding on the spores. Infected 3rd instars developed into adults in 11–15 days, but these adults often could not unfold their wings and were smaller in size than the controls. The ovaries of treated adults were heavily infected with the microsporidium and usually no eggs were present, except in two females, where one egg each was found. In contrast, uninfected adults averaged 100 eggs/female.

4. Discussion

Several experiments to find alternative control methods for the crucifer pest *H. undalis* have been conducted in Togo, Taiwan, and Malaysia (Dreyer,

Table 2
Spore size and tissue specificity of described *Vairimorpha* spp. infecting different lepidopteran hosts

Species	Spore size [μm] (length × width)[a]	Host	Tissue specificity	Author
Vairimorpha sp.	*di*: 3.55 (±0.29) 2.18 (±0.21)	*Hellula undalis* (F.)	General infection	Kleespies and Mewis (2001)
	oc: 2.44 (±0.20) × 1.64 (±0.14)			
Vairimorpha plodiae (Kellen and Lindegren)	*di*: 4.95 (±0.09) × 2.08 (±0.01)	*Plodia inter-punctella* (Hübner)	Primary midgut epithelium and fat body	Kellen and Lindegren (1968)
	oc: 2.27 (±0.02) × 1.60 (±0.01)			
Vairimorpha necatrix (Kramer)	*di*: 4.33 (±0.19) × 2.26 (±0.18)	*Spodoptera exempta* (Walker)	Fat body only	Pilley (1976)
	oc: 1.86 (±0.10) × 1.08 (±0.12)			
Vairimorpha ephestiae (Weiser and Purrini)	*di*: 4.0–4.3 × 2.0–2.5	*Ephestia kuehniella* (Zeller)	?	Weiser and Purrini (1985)
	oc: 2.5–3.0 × 1.5–2.0			
Vairimorpha sp.	*di*: 4.22 (±2.38) × 2.30 (±1.14)	*Plutella xylostella* (L.)	General infection	Nahif and Jungen (1986)
	oc: 3.45 (±1.96) × 2.08 (±1.17)			

[a] Spores measured on Giemsa-stained smears, *di*, diplokaryotic spores; *oc*, octospores.

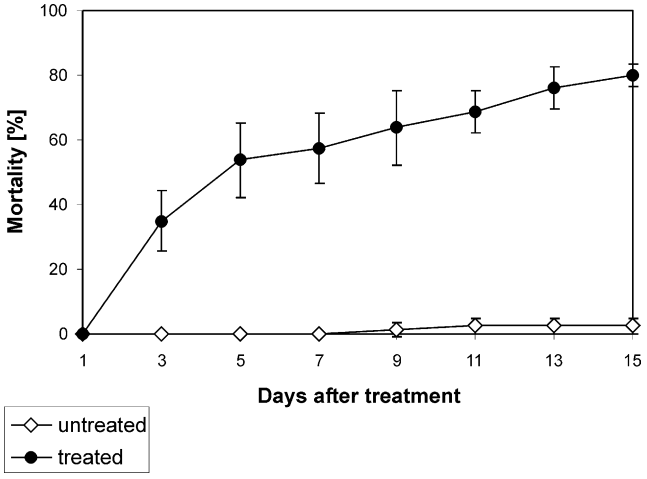

Fig. 2. Average mortality of *Hellula undalis* instars after feeding 3rd instar larvae on artificial diet treated with microsporidian spores or water (untreated) ($n = 40$, 4 repetition).

1987; AVRDC, 1987; Sivapragasam and Aziz, 1990). But the use of common less hazardous control agents such as Neem and *B. thuringiensis* was not sufficient. In our study we describe for the first time a microsporidian isolate of *H. undalis* in the Philippines. The microsporidium was established in the Philippine populations of *H. undalis* and may act as natural regulation factor. As no microsporidium has been previously described for *H. undalis*, this pathogen is of special interest for the possible use as microbiological control agent.

The microscope studies showed that the life cycle of the microsporidium of *H. undalis* includes a disporoblastic and an octosporoblastic phase, whereby binucleate free mature spores and packages of eight uninucleate octospores enclosed in a sporophorous vesicle, were produced, respectively. Such two discrete live cycle phases are the characteristics of the genus *Vairimorpha*, and for this reason the newly detected microsporidium of *H. undalis* was assigned to this genus. *Vairimorpha* spp. have been described from several other lepidopteran hosts, including the competing crucifer pest *P. xylostella*, but the spore sizes of the *H. undalis* microsporidium are smaller than in the described *Vairimorpha* sp. (Table 2). Furthermore, a general infection as observed in our investigations has only been found in *P. xylostella* infected with *Vairimorpha* sp. (Nahif and Jungen, 1986). However, the *P. xylostella* stock culture at CLSU-campus in the Philippines never became infected by the microsporidium of *H. undalis*, although the rearing of both insects took place in the same laboratory and no special quarantine for the *P. xylostella* rearing was performed. Additionally, no microsporidian infected *P. xylostella* was found in our field evaluation in the Philippines from 1998–2001. All these facts suggest that the microsporidium of *H. undalis* is a new species in the genus *Vairimorpha*.

In peroral infection experiments, the *Vairimorpha* sp. was highly infectious for *H. undalis*, whereby first mortalities of larvae were already observed on day three after exposure. Possibly invasions of saprophytic or facultatively pathogenic bacteria may have contributed to these early larval mortalities. However, such fast infections have also been described by Solter and Maddox (1998) for the host species *Spodoptera exigua* (Hübner) and *Lymantria dispar* (L.) when *Vairimorpha necatrix* (Kramer) and *Vairimorpha* sp. had been applied, respectively. Primary spores had been found in the midgut 36 h after spore injection into the hemolymph and mature environmental spores had been detected in the fat body tissues after 96 h, when larvae were kept under laboratory conditions at 24 °C. It should be mentioned that *H. undalis* can complete the life cycle in less than 12 days at temperatures of 30 °C and at 85% RH used for bioassays and rearing. High

temperatures and RH may favor a rapid dissemination of the microsporidium among insects.

According to our microscope studies and rearing observations, the microsporidium of *H. undalis* is obviously horizontally and probably vertically transmitted. After feeding of artificial diet with spores, 100% of *H. undalis* became infected, including the ovaries in emerged females. Additional evidence for vertical transmission is the observation that occasionally the offspring from isolated adults (originally obtained from the field) died due to the microsporidian infection (Mewis, unpublished data). Generally, this might be of importance in the possible use of infected adults in microbiological control. However, such use may be limited due to reduced fitness of emerged adults. The *Vairimorpha* sp. of *P. xylostella* is also passed transovarially between generations, but contrary to *H. undalis*, the infection had only little impact on the fitness of *P. xylostella* (Schuld et al., 1999). According to Krieg and Franz (1989) a high virulence, but not a hypervirulence and a good establishment in the pest population, are requirements for pathogens in microbiological control. The described *Vairimorpha* sp. of *H. undalis* seems to meet these requirements. Nevertheless, the host specificity to *H. undalis* has to be ascertained before a practical use of this microsporidian species in biological or integrated control can be considered. Other studies on the host range of *Nosema* and *Vairimorpha* microsporidia from Lepidoptera including infectivity tests to non-target organisms strengthen the assumption of a host specificity of many terrestrial microsporidia (Solter and Maddox, 1998; Solter et al., 2000).

The employment of microsporidia in microbiological control implies some problems and limitations: (1) the mass rearing is only possible in the living host; (2) the spore formula and field application is problematic, and (3) there is no immediate mortality, thus larvae can damage the plant before death. Furthermore, vectoring of a pathogen by ovipositing parasitoids is a frequently occurring phenomenon and this can negatively affect the performance of parasitoids as described for the host–parasitoid–pathogen system *L. dispar—Glyptapanteles liparidis* (Bouché) and—*Vairimorpha* sp. (Hoch et al., 2000). Consequently, the employment of microsporidia as control agents may limit the additional use or control capacity of parasitoid antagonists.

In summary, the present results encourage further studies to evaluate the possible use and the potential of *Vairimorpha* sp. in a control program against *H. undalis*. However, further infection experiments in the laboratory and in the fields have to ascertain the efficacy and profitability of this pathogen. The final taxonomic assignment of the microsporidium of *H. undalis* depends on the results of further studies on morphological, ultrastructural, and genetic studies as well as infection tests on the host range.

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References

AVRDC, 1987. Screening Chinese cabbage accessions for resistance to cabbage webworm. Progress Report, 1985. Asian Vegetable Research and Development Center, Shanhua, Taiwan, pp. 19–23.

Battu, G.S., 1991. Yield levels of the occlusion bodies obtained from the baculovirus-infested *Hellula undalis* Fabricius. Indian J. Entomol. 53, 520–522.

David, L., Weiser, J., 1994. Role of hemocytes in the propagation of a microsporidian infection in larvae of *Galleria mellonella*. J. Invertebr. Pathol. 63, 212–213.

Dreyer, M., 1987. Field and laboratory trials with simple neem products as protectants against pests of vegetables and field crops in Togo. In: Schmutterer, H., Ascher, K.R.S. (Eds.), Natural pesticides from the neem tree (*Azadirachta indica* A. Juss) and other tropical plants. Proceedings of the 3rd International Neem Conference, Nairobi, Kenya, Schriften der Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn, Germany, pp. 431–447.

Henry, J.E., Oma, E.A., 1981. Pest control by *Nosema locustae*, a pathogen of grasshoppers and crickets. In: Burges, H.D. (Ed.), Microbial Control of Pests and Plant Diseases 1970–1980. Academic Press, New York, pp. 573–586.

Hoch, G., Schopf, A., Maddox, J.V., 2000. Interactions between an entomopathogenic microsporidium and the endoparasitoid *Glyptapanteles liparidis* within their host, the gypsy moth larva. J. Invertebr. Pathol. 75, 59–68.

Kellen, W.R., Lindegren, J.E., 1968. Biology of *Nosema plodiae* sp. n., a microsporidian pathogen of the Indian meal moth, *Plodia interpunctella* (Hübner), (Lep.: Phycitidae). J. Invertebr. Pathol. 11, 104–111.

Kleespies, R.G., Mewis, I., 2001. Light and electron microscope studies on a microsporidium of the cabbage webworm, *Hellula undalis* (F.) and epizootiological studies in the Philippines. Abstracts of the 34th Annual Meeting of the Society for Invertebrate Pathology, Noordwijkerhout, Netherlands, August 25–30, p. 434.

Krieg, A., Franz, J.M., 1989. Lehrbuch der Biologischen Schädlingsbekämpfung. Verlag Paul Parey, Berlin und Hamburg.

Kurtz, J., Nahif, A.A., Sauer, K.P., 2000. Phagocytosis of *Vairimorpha* sp. (Microsporida, Nosematidae) spores by *Plutella xylostella* and *Panorpa vulgaris* hemocytes. J. Invertebr. Pathol. 75, 237–239.

Mewis, I., Ulrichs, Ch., Schnitzler, W.H., 2001a. Feeding behavior and host-plant finding of the cabbage webworm *Hellula undalis* (Fabricius) (Lepidoptera: Pyralidae) in the context of secondary host-plant compounds. Mitt. Dtsch. Ges. all. angew Entomol. 13, 273–278.

Mewis, I., Ulrichs, Ch., Schnitzler, W.H., 2001b. Oviposition preference of the cabbage webworm *Hellula undalis* (Fabricius) (Lepidoptera: Pyralidae). Mitt. Dtsch. Ges. all. angew. Entomol. 13, 535–538.

Moser, B.A., Becnel, J.J., Williams, D.F., 2000. Morphological and molecular characterization of the *Thelohania solenopsae* complex (Microsporidia: Thelohaniidae). J. Invertebr. Pathol. 75, 174–177.

Nahif, A.A., Jungen, C., 1986. Morphology and development of *Vairimorpha* sp. (Microsporida, Nosematidae) from diamondback

moth *Plutella xylostella* (Lepidoptera, Yponomeutidae). J. Appl. Entomol. 122, 617–621.

Onstad, D.W., Carruthers, R.I., 1990. Epizootiological models of insect diseases. Annu. Rev. Entomol. 35, 399–419.

Pilley, B.M., 1976. A new genus, *Vairimorpha* (Protozoa: Microsporida), for *Nosema necatrix* Kramer 1965: pathogenicity and life cycle in *Spodoptera exempta* (Lepidoptera: Noctuidae). J. Invertebr. Pathol. 28, 177–183.

Rejesus, B.M., Javier, P.A., 1997. Assessment of the occurrence and abundance of *Hellula undalis* in the Philippines. AVNET-II Final Workshop Proceedings (AVRDC), pp. 314–320.

Schuld, M., Madel, G., Schmuck, R., 1999. Impact of *Vairimorpha* sp. (Microsporidia: Burenellidae) on *Trichogramma chilonis* (Hymenoptera, Trichogrammatidae), a hymenopteran parasitoid of the cabbage moth, *Plutella xylostella* (Lepidoptera, Yponomeutidae). J. Invertebr. Pathol. 74, 120–126.

Sivapragasam, A., Aziz, A.M.A., 1990. Cabbage webworm on crucifers in Malaysia. In: Talekar, N.S. (Ed.), Diamondback Moth and Other Crucifer Pests. Asian Vegetable Research and Development Center, pp. 75–80. (Chapter 8).

Sivapragasam, A., Ruwaidah, M., Asma, A., 1994. Temperature and its influence on the development and distribution of *Hellula undalis* in Malaysia. In: Rajan, A., Yusof-Ibrahim, (Eds.), Plant protection in the tropics: proceedings of Fourth International Conference on Plant Protection in the Tropics. Kuala Lumpur (Malaysia), vol. 5. Malaysian Plant Prot. Soc, pp. 56–58.

Sivapragasam, A., Chua, T.H., 1997a. Preference for sites within plant by larvae of the cabbage webworm, *Hellula undalis* (Fabr.) (Lep., Pyralidae). J. Appl. Entomol. 121, 361–365.

Sivapragasam, A., Chua, T.H., 1997b. Natural enemies for the cabbage webworm, *Hellula undalis* (Fabr.) (Lepidoptera: Pyralidae) in Malaysia. Res. Popul. Ecol. 39, 3–10.

Solter, L.F., Maddox, J.V., McManus, M.L., 1997. Host specificity of microsporidia (Protista: Microspora) from European populations of *Lymantria dispar* (Lepidoptera: Lymantriidae) to indigenous North American Lepidoptera. J. Invertebr. Pathol. 69, 135–150.

Solter, L.F., Maddox, J.V., 1998. Timing of an early sporulation sequence of microsporidia in the genus *Vairimorpha* (Microsporidia: Burenellidae). J. Invertebr. Pathol. 72, 323–329.

Solter, L.F., Pilarska, D.K., Vossbrinck, C.F., 2000. Host specificity of microsporidia pathogenic to forest Lepidoptera. Biol. Contr. 19, 48–56.

Srihari, D., Satyanarayana, M., 1992. Evaluation of some exotic cabbage F1 hybride and cultivares. South Indian Hort. 40, 28–33.

SYSTAT 9.0 Handbook, 1999. SYSTAT Statistics. SPSS Science, Chicago, Statistics. vol. 1, 660 pp.

Tanada, Y., Kaya, H.K., 1993. Protozoan infections: Apicomplexa, Microspora. In: Tanada, Y., Kaya, H.K. (Eds.), Insect Pathology. Academic Press, San Diego, pp. 414–458.

Waterhouse, D.F., Norris, K.R., 1989. Biological Control Pacific Prospects Supplement 1. Australian Centre for International Agricultural Research, Canberra, pp. 77–81.

Weiser, J., Purrini, K., 1985. Light an electron microscopic studies on the microsporidian *Vairimorpha ephestiae* (Mattes) (Protozoa, Microsporida) in the meal moth *Ephestia kuehniella*. Arch. Protistenk. 130, 179–189.